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- (54) **TREATING NEURODEGENERATIVE DISEASES WITH GGA OR A DERIVATIVE THEREOF**
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Primary Examiner — Kathrien Cruz(74) *Attorney, Agent, or Firm* — Foley & Lardner LLP(57) **ABSTRACT**

Provided herein are methods for improving negative effects of ALS, AD, or ischemia, the methods comprising administering to a subject in need thereof a therapeutically effective amount of GGA, preferably present in the trans form, or a derivative thereof.

1 Claim, No Drawings

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TREATING NEURODEGENERATIVE DISEASES WITH GGA OR A DERIVATIVE THEREOF

CROSS REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. provisional application No. 61/711,162, filed Oct. 8, 2012, the contents of which is included herein in its entirety by references.

FILED OF INVENTION

This invention relates to improving negative effects of amyotrophic lateral sclerosis (ALS), alzheimers disease (AD), or ischemia by administering geranylgeranyl acetone (GGA) or a derivative.

STATE OF THE ART

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder that causes muscle weakness, disability, and eventual death, with a median survival of three to five years.

Incidence rates for ALS in Europe and North America range between 1.5 and 2.7 per 100,000/year, while prevalence rates range between 2.7 and 7.4 per 100,000; there is no definite racial or ethnic predisposition. Overall, the male to female ratio is about 1.3 to 1.5, with higher male predominance at younger age and near equality at older ages. The incidence of ALS increases with each decade, especially after age 40 years, and peaks in the seventh to eighth decade. In the United States, about 7000 new cases of ALS are diagnosed each year. The incidence and mortality rates of ALS have been slowly increasing over decades. Part or all of this increase in ALS incidence may be due to longer life expectancy.

Worldwide, approximately 90% of cases of ALS are sporadic (SALS), with the remaining 10% familial (FALS) and caused by mutations in one of several genes. SALS is likely linked to alterations in complex gene systems, forming genetic risks factors rather than a direct cause of ALS. In FALS there is often Mendelian inheritance and high penetrance, with most cases following an autosomal dominant inheritance pattern.

The genetic etiology of nearly half of FALS is unknown. Approximately 15 genetic mutations have been identified as causes of ALS, although the causative nature of the gene mutations is still unknown. Among these, mutations in 72C9ORF72, SOD1, TDP43, FUS, ANG, VCP, OPTN are associated with typical ALS; the remainder are associated with unusual phenotypes or small numbers of kindreds. The most common mutation appears to be C9ORF72 (FTD-ALS) repeat expansion accounting for 24 to 47% of FALS, while the second most common gene mutation, SOD1, accounts for about 12% to 20% of FALS. The most common mutation in North America is the A4V, which is associated with a very aggressive form of ALS accounting for 50% of SOD1 patients.

The central pathology of ALS is atrophy and death of anterior horn cells and cortical motor neurons, and may affect other neuronal populations, particularly frontal cortical neurons, leading to concurrent frontotemporal dementia. Differences in site and segment (cranial, cervical, thoracic, or lumbosacral) of onset, pattern and speed of spread, and the degree of upper and/or lower motor neuron dysfunction produce a disorder that is remarkably variable between individuals. The

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initial clinical manifestation of ALS may occur in any body segment (bulbar, cervical, thoracic or lumbosacral) and may manifest as upper motor neuron or lower motor neuron symptoms or signs. Asymmetric limb weakness is the most common presentation of ALS (80%), followed by bulbar onset, respiratory muscle weakness, or generalized weakness. The frequency of frontotemporal executive dysfunction in patients with ALS reportedly occurs in 35-51% of patients with ALS. The median survival from the time of diagnosis is three to five years; however, about 10% of patients live 10 years or more. A principal difficulty in the diagnosis and management of ALS is the absence of clinical biomarkers, which contributes to a diagnostic delay of 9 to 12 months and to initial false-negative diagnosis in up to 10% of cases.

The complex pathophysiology of ALS suggests that it is a multifactorial disorder and presents many potential therapeutic targets. In recent years, a large number of experimental therapies and neuroprotective agents with varying mechanisms of action have been tested clinically (including agents targeting excitotoxicity, neurotrophic agents, immunomodulators, agents targeting oxidative stress, anti-apoptotic compounds and nutritional supplements) and have failed. Although several drugs (such as creatine, celecoxib, IGF-1, CNF, gabapentin, topiramate, lamotrigine, xaliproden, minocycline, thalidomide, valproate, vitamin E) gave positive results in preclinical animal studies, none of these compounds, when tested in humans, significantly prolonged survival or improved quality of life of ALS patients.

A range of mechanisms have been implicated in the initiation and propagation of the neurodegenerative process in ALS that may not be mutually exclusive and include excitotoxicity, oxidative stress due to free radical generation, protein misfolding, protein aggregation, neurofilament aggregation, mitochondrial dysfunction, proteasomal dysfunction, aberrant growth factor signaling, apoptosis, impaired axonal transport, microinflammatory process and glial activation.

Numerous agents with potential neuroprotective effects that have been investigated include those from the categories of free radical scavengers, anti-excitotoxic agents, apoptosis inhibitors, anti-inflammatory agents, neurotrophin factors, metal ion chelators, ion channel modulators and gene therapies.

Two preclinical and clinical strategies in ALS drug development are: 1) preventing death of the motor neuron soma by pharmacologically targeting the neuron and 2) supporting motor neuron health indirectly by targeting the biology of ambient glia. A third strategy gaining momentum in preclinical studies reconceptualizes ALS as a cytoskeletal disease and targets degenerative processes involving neuromuscular junctions (NMJs). It is based on the fact that motor neuron cell death only happens near the end of an extensive clinical disease and thus likely is a final consequence of other neuronal processes, whereas NMJ damage occurs in the early stages of disease progression, long before motor neuron loss. ALS can be thought of as a progressive distal axonopathy that follows NMJ denervation but precedes both neuronal degeneration and the onset of clinical symptoms.

Recently, several new agents have reportedly shown evidence of benefit in Phase 2 trials. Particularly, dexamiprexole, an amino-benzothiazole, through actions involving mitochondria, tirasemtiv (CK-2017357), a fast skeletal muscle troponin activator, and NP001, a regulator of macrophage function.

Only one therapeutic with similar targets has been tested in clinical trials for ALS. Arimoclomol is a drug that upregulates molecular chaperones in cells. Pre-symptomatic administration as well as treatment with arimoclomol at disease onset

extended the life span of SOD1 G93A by approximately 5 weeks. In a Phase 2a open label clinical trial run, a reduction in decrease of ALSFRS-R, vital capacity, total body weight and body mass index was observed when compared with a historical control. No other clinical trial has been performed investigating HSP70 induction or modulation of GGTase in ALS.

Despite over 30 phase II and phase III clinical trials of promising agents, riluzole remains the only evidence-based disease-modifying drug for ALS. An American Academy of Neurology practice guideline, published in 2009, recommended riluzole, a benzothiazole analog, as safe and effective for the slowing of progression in ALS, though only to a modest degree and without effect on motor function. The gain in survival is approximately 11% or 3 months. The precise mode of action of riluzole is unknown. It appears to be a low potency, nonspecific modulator of multiple pharmaceutical targets that include the inhibition of presynaptic glutamate release, inactivation of voltage-dependent ion channels, prevention of protein aggregation, and more recently, neuroprotection via an astrocyte dependent mechanism leading to enhanced glutamate reuptake rather than by regulating glutamate release. Due to the inevitably progressive nature of ALS, symptomatic, preservation of quality of life, and palliative treatments contribute significantly to clinical management of patients with ALS, regardless of riluzole treatment.

Extension of survival over that achieved by riluzole is considered a minimally acceptable result that must be accompanied by a slowing of disease progression, as measured by comparing pretreatment and 6-12 months of treatment ALSFRS-R scores, and that delays onset of ventilator assistance in both familial and sporadic ALS. Also, as a minimum, the drug must be safe and tolerable to the majority of eligible patients. The drug would be taken twice daily mixed in food or drink with little to no pharmacokinetic interference.

The upregulation of HSP70, for example, has been reported with riluzole and arimoclomol. HSP70 is effective with its co-chaperone HSP40 that features a CAAX prenylation motif and which is required for binding of GGT. Pleiotropic effects of these drugs, can be observed although in most, the mechanism of action has not been determined.

Modulation of prenylation can also be a part of the mechanism of action of statins and bisphosphonates. These drugs block the cholesterol synthesis pathway, an intermediate of which is GGPP, the main substrate of GGTase. Inhibition of prenylation has been invoked as an explanation of the anti-inflammatory effects of statins and it is conceivable that this results in neuroprotection in ALS. Furthermore it has been reported that statins are able to improve clearance of misfolded proteins in a model of Alzheimer's disease by modifying prenylation. However, to our knowledge, these pharmaceutical approaches have not been investigated for drug development in ALS.

SUMMARY OF INVENTION

Provided herein are methods for improving negative effects of ALS, AD, or ischemia, the methods comprising administering to a subject in need thereof a therapeutically effective amount of GGA, preferably present in the trans form, or a derivative thereof. As used herein, a therapeutically effective amount refers to an amount of GGA or a derivative thereof that can improve the negative effects of ALS, AD, or ischemia in a subject or an in vitro or in vivo model thereof. As also used herein, the term "treatment" or "treating" means any treatment of a disease or condition in a patient, including one or more of:

preventing or protecting against the disease or condition, that is, causing the clinical symptoms not to develop, for example, in a subject at risk of suffering from such a disease or condition, thereby substantially averting onset of the disease or condition;

inhibiting the disease or condition, that is, arresting or suppressing the development of clinical symptoms; and/or

relieving the disease or condition that is, causing the regression of clinical symptoms.

Modes of administering an agent, such as GGA or a derivative thereof, in accordance with the present invention, is well known to the skilled artisan and/or will be apparent to the skilled artisan upon reading this disclosure. In a preferred embodiment, the GGA or the derivative thereof is administered as a 7 day patch.

DETAILED DESCRIPTION OF THE INVENTION

Provided herein are methods related to modulation of the activity of small GTPases by influencing their prenylation to stabilize the cytoskeleton in neurites for the treatment of ALS. In some embodiments, these methods utilize CNS-102, which is a structural mimic of the prenylation substrate GGPP. Prenylation influences multiple signaling pathways that modulate functions such as cell movement, motility, cell growth, cell survival, and expression of HSPs.

In some embodiments, the compounds utilized herein extend survival and improve quality of life by inhibiting the disease progression and demonstrate improvement in the slope of the ALSFRS-R scores measured over a 6-12 month period. In some embodiments, the compounds utilized herein are administered once daily in food or drink, or delivered by a 7-day patch.

In some embodiments, the compounds utilized herein have the following chemical properties for optimal CNS penetration and bioavailability: molecular weight <400, lack ionization at physiological pH, lipophilic, polar surface area estimated at 60-90 Å², minimal hepatic first pass effect, i.e., no significant CYP2D6 metabolism. Additionally, the compound is well absorbed and have high absolute oral bioavailability in order to minimize the total amount of drug needed and to attenuate side effects or interactions with other drugs. Additionally advantages would include minimal compound that is bound to plasma proteins over the clinical concentration range; pharmacokinetics that are linear over the therapeutic dose range, and PK given every 24 hours for effective single daily dosing.

Embodiments including certain compound characteristics are tabulated below.

Compound Properties	Minimum Acceptable Result	Ideal Result
Primary Drug Indication	Treatment to extend survival, slow progression and delay onset of ventilator assistance in sporadic and familial ALS	Treatment to extend survival and halt neurodegeneration in sporadic and familial ALS
Patient Population	Adults with ALS	Adults with ALS
Delivery Mode	Oral	Oral or Patch
Treatment Duration	Chronic	Chronic
Regimen	2x/day	1x/day oral or 1x/week patch

-continued

Compound Properties	Minimum Acceptable Result	Ideal Result
Efficacy	Extends survival by $\geq 20\%$, slows progression as measured by the slope of ALSFRS-R and delays onset of ventilator assistance in sporadic and familial ALS. Safe and well tolerated	Extends survival $\geq 40\%$ and delays onset of ventilator assistance. Inhibits neurodegeneration and stabilizes the disease. Demonstrates improvement in slope of ALSFRS-R over a 9-month period. Safe and well tolerated

In these regards, 5E,9E,13E-geranylgeranylacetone (trans GGA or CNS-102), which is an isomorph of geranylgeranyl pyrophosphate (GGPP), the prenylation substrate for geranylgeranyl transferase, (GGTase I) exhibits neuroprotective activity capable of supporting different classes of neurons threatened by a variety of endogenous genetic or exogenous pharmacologic stressors. In vitro, CNS-102 protects neurites from toxicity caused by an inhibitor of GGTase I. Systemic CNS-102 upregulates expression of several HSP genes in the brain cortex and hippocampus, with concomitant induction of HSP expression in brain cortex. It also provides neuroprotection in the hippocampus against excitotoxicity induced by kainic acid. In the superoxide dismutase 1 (SOD1) mouse model of ALS, CNS-102 significantly prolonged survival, improving clinical scores, neurological scores, and computerized assessment of gait parameters, suggesting that CNS-102 can also be effective in delaying onset or progression of symptoms.

It is contemplated that that compounds such as CNS-102 and analogs based on its scaffold can modulate geranylgeranyltransferase I (GGTase I) activity. This in turn is thought to regulate prenylation of small G-proteins such as Rho, Rac and Cdc42 and affect their activities on various aspects of neuronal development, including neurite outgrowth and differentiation, axon pathfinding, and dendritic spine formation and maintenance. Furthermore, it is contemplated that compounds based on the CNS-102 scaffold can induce HSP expression such as HSP70, and lead to inhibition of cytotoxicity-induced neuronal cell death during the pathological process of ALS. In some embodiments, treatment with CNS-102 or analogs may provide neuroprotection by inhibiting apoptosis and promoting regeneration of degenerating motor neurons, thus improving survival and behavioral outcome measures of ALS patients. Moreover, since some of the aforementioned molecular and cellular pathological features of ALS are shared by AD and HD patients, it is contemplated that our compounds can also benefit the treatments of other neurodegenerative diseases.

A hallmark feature of ALS is muscle weakness caused by denervation, dysfunction and degeneration of motor neurons. While other muscle types respond to stress and damages by up-regulation of heat shock proteins such as HSP70, motor neurons are lacking the response of HSP70 upregulation, probably rendering them more vulnerable to a variety of insults.

A key component of the proposed mechanism of action of CNS-102 is to up-regulate HSP70 activity, therefore inducing a cytoprotective and anti-apoptotic response in neurons. Post-translational protein prenylation mediated by GGTase I, regulates activities of small GTPases such as Ras, Rap1, and Rho (Rho, Rac, Cdc42), modulating a signal cascade that leads to activation of HSP70. These GTPases are involved in multiple cellular processes, including proliferation, apoptosis, cell morphogenesis and metastasis in a multitude of tissues.

GGTase I is one of the two major enzymes that catalyze the attachments of isoprenoid lipids to the C-termini carrying CaaX motif of proteins. Despite some reports that GGTase I is dispensable for membrane association and/or forming GTP-bound small GTPases in non-neuronal cells, it is considered that GGTase I dependent prenylation is required for activating Rho GTPases at the cell membrane in neuronal cells. As an example, one strategy for modulating Rho GTPase activities has been the inhibition of GGTase I in the treatment of cancer.

GGA has been reported to be an inducer of HSP70 activity conferring neuroprotection in vitro in models of Parkinson's disease and heat injury such as burns. GGA also proved to be neuroprotective in vivo by inducing HSP70 in models of ischemia and glaucoma. In a model of proteasome impairment in cultured motor neurons, simulating conditions that may be found in ALS, GGA induced the expression of HSP70 and was found to be neuroprotective. Recently, it was shown in motor neurons of the SOD1 G93A model that a co-inducer of the heat shock response, arimoclocholol, increased innervation, end plate size, and activity of cholinergic enzymes and reversed muscle fiber transformation, compared to control groups. This was accompanied by a concomitant increase of HSP70 expression. Interestingly, injection of human recombinant HSP70 protein in SOD1 mice delayed the peripheral muscle denervation.

Upregulation of HSP70, a measurable outcome of prenylation, has been demonstrated to be beneficial in several models of neurodegeneration and also in vivo in models of motor neuron disease and in mouse models of ALS. In some embodiments, treatment with CNS-102 analogs improve several outcome measures (life span, motor performance, muscle strength, motor neuron denervation, neuropathology in brain and spinal cord) in the SOD1 G93A mouse model of ALS. CNS-102 shows efficacy at nanomolar levels and exhibits excellent bioavailability and brain penetration, which makes it a suitable candidate for treatment in human patients with ALS.

Biomarkers

Without being bound by theory, the mechanism of action of CNS-102 is postulated to be prenylation of G proteins and induction of HSPs. It has been determined that in murine Neuro2A cells and in a rat kainic acid (KA) induced neurotoxicity model, CNS-102 increases expression of HSPs. Expression of HSP70 and HSP90 was found in the neuroblastoma cell study, while in the neuroprotection study, increased expression of HSP70 in hippocampus and cerebral cortex was observed in addition to upregulation of HSP60, HSP70, and HSP90.

Since a therapeutic effects of CNS-102 in ALS relate to intracellular actions of HSPs, changes in intracellular levels of HSP70 can be relevant as a biomarker along with HSP27 and HSP90. Circulating white blood cells are the most conveniently accessible cells and two small clinical trials have reported transient induction of HSP70 and HSP90 in PBMCs following a single dose of geranylgeranylacetone (GGA) or a GGA derivative. HSP70 is also detectable in cerebrospinal fluid (CSF) where it presumably is derived from brain and spinal cord tissue. Elevation has been reported in relationship to ischemia and seizure. CSF HSP70 is advantageous in that changes are more likely to reflect events in the CNS, but would only be considered if a suitable peripheral biomarker could not be established. Studies with ex vivo human PBMCs, would determine if changes in PBMC HSP are detectable following drug exposure, and if these correlate with changes in CNS HSP expression. If HSP induction is

insufficient in PBMC, skin or muscle can serve as alternative tissue sources for assessment of HSP induction in clinical trials. Events in skeletal muscle may be of greater relevance for ALS, since increasing muscle HSP70 correlated to rescue of SOD1 mice. In some embodiments, both skin and muscle are less attractive than blood because biopsy is inherently more invasive than phlebotomy, increasing discomfort for subjects and usually limiting sampling to one or two post-treatment time points.

Preclinical Studies

The following experimental evidence support CNS-102's activity as a neuroprotective agent capable of protecting different classes of neurons threatened by a variety of endogenous genetic or exogenous pharmacologic stressors. 1) In vitro, CNS-102 protects neurites on neuroblastoma cells from toxicity caused by an inhibitor of geranylgeranyl transferase. 2) Systemic CNS-102 has also provided neuroprotection in the hippocampus against locally injected kainic acid. This benefit is prolonged with peak protection at 72 hours following a single oral dose. 3) CNS-102 significantly prolonged survival in the SOD1 mouse model of ALS and improved clinical scores, neurological scores, and computerized assessment of gait parameters reaching statistical superiority over scores in vehicle treated mice. Successful prolongation of survival with CNS-102 in this well recognized murine model of ALS is the chief justification for the proposed use of CNS-102 as a lead compound for the indication of ALS.

In the SOD1 mouse model of ALS (N=16/treatment, randomized design), daily treatments of CNS-102 and riluzole each produced a statistically significant improvement in median survival time compared to the vehicle control group, and CNS-102 outperformed riluzole by 50%, although the difference between the two drug treatment groups, when compared directly, was not statistically significant at $p=0.05$. Analyses were conducted using PROC LIFEREG in SAS.

Group	Increase over vehicle	p-value
CNS-102	9%	0.0007
riluzole	6%	0.0149

Additionally, three neurological function outcomes were chosen for analysis from those measured on a 'cat-walk' at irregular intervals over the duration of the study; stride length (cm), run duration (s), and swing speed (cm/s). Prior to post-natal day (P) 119, there were only small differences between treatment group averages. Two specific subsets of data were collected for all of the surviving animals: one over a 2-day period from P119 to P121 (21 animals; 12 CNS-102, 9 vehicle), and the other over the interval of P122 and P127 (13 animals; 10 CNS-102, 3 vehicle).

By P119, the progression of the disease had both increased the measured differences in averages between treatments and removed significant but unequal fractions of each treatment group. This non-random censoring of the treatment group data precluded using standard comparative statistical methods such as the t-test due the unverifiable compliance of the remaining data with required statistical test assumptions. Consequently, a Monte Carlo estimate of the p-value of the treatment differences was constructed using the bootstrap method. The p-value for each difference in drug-minus-vehicle averages is calculated by comparison to the distribution produced from bootstrap results of 10 MM iterations. These are shown in the table below.

Comparison of Averages				
Age	P120 \pm 1		P124 \pm 3	
Metric	CNS-102-vehicle	p-value	CNS-102-vehicle	p-value
Stride Length	1.46	0.031	1.87	0.0062
Run Duration	-1.68	0.034	-1.49	0.0077
Swing Speed	14.58	0.017	11.70	0.0206

Furthermore, these p-values did not suffer significant change when the minus-one jackknife procedure of Quenouille and Tukey was applied to the data. This demonstrated that no single animal unduly influenced the construction of the reference distribution, thereby bolstering confidence in these p-value estimates.

These results are taken as proof that CNS-102 has the activity required for a medicinal chemistry synthesis program, and the scaffold from which CNS-102 is constructed is a valid framework from which to propose new structures. Clearly, the fact that all three neurological metrics show significantly better outcomes compared to vehicle, indicates that CNS-102 not only prolongs survival but also slows the symptomatic progression of the disease.

Primary Screening Assay—HSP70 Expression

It is contemplated that compounds useful according to the present methods can be identified by screening pools of compounds for their effect on prenylation. It has been found that CNS-102 I modulates GGTase I activity as detected by a fluorescent peptide, dansyl-GCVLL. CNS-102 treatment can lead to increased prenylation of important signaling molecules, whose activation in turn results in unregulation of HSP protein expression. In some embodiments, increase in HSP70 is chosen as the primary screening assay target for identifying GGA derivatives particularly suitable for the methods provided herein.

HSP70 expression in neuro2A cells treated with CNS-102 analogs are examined, using Western blot analysis. An increased expression of HSP70 will indicate the candidate molecule has the prerequisite activity. This assay has a Z factor of -0.9 and a CV of 11% for CNS-102 at 1 nM to 100 nM.

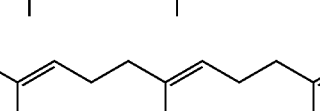
A commercial GGA related compound, teprenone, which preserves motor neurons, has been shown to regulate HSP expression at the level of heat shock transcription factor 1 (HSF1). It can bind to HSP70 to relieve the inhibition of HSF1 and allow the latter to translocate to nucleus and activates new HSP proteins. It is contemplated that the consequence of increased HSF1 nuclear translocation upon CNS-102 treatment, notably the upregulation of HSP70, can be observed.


Secondary Assay—Neurite Outgrowth

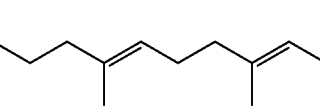
Neurite outgrowth in neuro2A neuroblastoma cell lines has been widely used as a cellular model for compounds screening in the CNS drug discovery and research. In some embodiments, the effects of CNS-102 analogs on neurite outgrowth is determined. Cells will be pre-treated with candidates and then subjected to a challenge with GGTase I inhibitor GGTI-298. Retinoic Acid will be added to start the differentiation of these cells. This will identify analogs that protect neurite outgrowth, which is known to be important for establishing synaptic connections and neuronal functions. This assay has a Z factor of 0.4 and a CV of 11% for CNS-102 at 10 nM to 1 μ M.

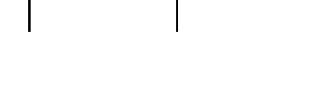
Survival studies and behavioral tests will be performed in the SOD1 G93A model of ALS. This transgenic mouse harbors a point mutation (G93A), found in familial ALS, in the gene encoding for superoxide dismutase 1 (SOD1). This very well characterized mouse model is widely used in ALS research. SOD1 G93A mice exhibit all of the histopathological hallmarks of familial and sporadic ALS that are observed in human patients. Motor neuron death in the ventral horn of the spinal cord and loss of myelinated axons in the ventral motor roots lead to paralysis and muscle wasting. Mice initially develop normally and gain weight until approximately 100 days of age. At this point, weight loss (which is usually defined as the onset of disease), muscle weakness, and gradual paralysis start at the hind limbs and muscle wasting start to appear. Maximum median life span is usually between 129-170 days depending on the background strain. This means that drug efficacy studies using this model can be performed within a relatively short time frame of 4-5 months. To rigorously test the efficacy of candidates in this mouse


The chemical structure of CNS-102 is shown. We have characterized CNS-102 (MW 330) by ¹H NMR, LC, and ESI mass spectrometry and no evidence of cis isomer was found. CNS-102 is a colorless oil with a density of 0.87 g/mL, having a calculated topological polar surface area (tPSA) of 17.07 and a calculated Log P of 7.61. Because CNS-102 is lipophilic, it has little intrinsic water solubility. Such can enable CNS-102 to cross the blood brain barrier.


 CNS-102


 CNS-G001

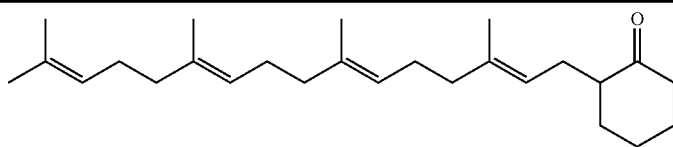

 CNS-G002


 CNS-G003


 CNS-G004

-continued

Structures Tested Based on CNS-102 Scaffold



CNS-G005

Screening Strategy and Bioactivity Assays

A preliminary screen and quantitative performance evaluation, and a confirmatory neuroprotective assay are developed from a model of motor neuron dysfunction in a PNS cell line. The screen and initial quantitative performance evaluation is conducted on a single biomarker, HSP70, which has been found to be broadly implicated to neuroprotection based on the rationale that induction of HSP70 is a necessary property of the potential treatment for ALS.

The overall strategy, therefore, is to use a sequence of assays, each of which is targeted to address a specific aspect of the disease with the goal of identifying candidates having multiple therapeutic capabilities. These assays are chosen such that a failure to perform at any stage is expected to be fatal to the candidate's suitability as a viable ALS treatment. To this end, the following four therapeutic capabilities and corresponding assays were chosen as shown in the list below. The advancement criteria, as well as assay design where relevant, are also presented.

1. In some embodiments, the candidates increase HSP70 expression in target cells. The initial screen (pass/fail, Assay 1a) and quantitative evaluation (EC_{50} , Assay 1b) of candidates will use a HSP70 expression assay by Western blot on cellular extracts of treated Neuro2A cells. The screen, Assay 1a, is conducted at a single concentration, requiring a specific level of activity to pass into Assay 1b, the quantitative dose/response determination of the EC_{50} . Only those candidates having an EC_{50} below 10 nM would be promoted to Assay 2.
2. In some embodiments, the candidates increase neuronal cell health/growth in the presence of specific inhibitors of GGTase I. The ability of candidates to promote neurite extension will be measured on Neuro2A cells to model whole-cell activities important to synaptic connections and neuronal function. Candidates showing >200 cells with outgrowth would be promoted to Assay 3.
3. In some embodiments, the candidates provide protection to nerve tissue from excitotoxins. Neuroprotection afforded by candidates that have passed the first two assays will be determined on rat hippocampal neurons facing kainic acid induced damage. This is the final animal model before the ALS disease model and candidates that provide >50% protection would be tested in the disease model.
4. In some embodiments, the candidates increase median survival time by 25% and improve neurological performance compared to control. This confirmation will be determined in the SOD1 mouse model.

Bioactivity Assays

#	Assay	Throughput	Advancement Criteria
1a	HSP70 Expression	30/ per 2 weeks	Promote if $\geq 1.5X$ PBS at 1 μM
1b			$EC_{50} \leq 10$ nM
2	Neurite Outgrowth	20 per 2 weeks	Promote if ≥ 200 cells at 10 nM
3	Rat Neuroprotection Model	8-12 per month	Promote if $\geq 50\%$ protection at 2 or 20 mg/kg
4	SOD1 Mouse ALS Model	3 per 6 months	25% increase in survival. Improved clinical and neurological scores.

In some embodiments, the assays were arranged in a specific order to maximize the early throughput with minimal effort being expended on the more labor-intensive assays, which maximizes overall throughput. This organization provides a "fail-early" probability to those compounds that have lower likelihood of clinical success, which removes them as early as possible from further consideration. Those candidates that pass through the assay cascade will be tested in the SOD1 mouse ALS model for confirmation.

Correlation of Assay Cascade Results

Assay	1a & 1b	2	3	4
Name	HSP70 Expression	Neurite Outgrowth	Rat Neuroprotection	SOD1 Mouse Model
Metric	HSP70 by Western Blot	Cell count relative to negative control	% Protection	Survival and clinical scores
Promotion Criteria	$\geq 1.50 \times$ PBS	≥ 2.00 @ 10 nM	$\geq 75\%$	n/a
CNS-102	$2.05 \times$ PBS	2.00	40%	9% increase in survival
CNS-G001	$2.00 \times$ PBS	1.79	37%	n/a
CNS-G002	$1.26 \times$ PBS	1.79	0%	n/a
CNS-G003	$2.78 \times$ PBS	2.74	82%	n/a
CNS-G004	$2.34 \times$ PBS	2.61	76%	n/a
CNS-G005	$2.78 \times$ PBS	2.28	83%	n/a
Correlation	n/a	78%	86%	n/a

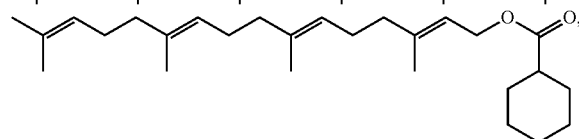
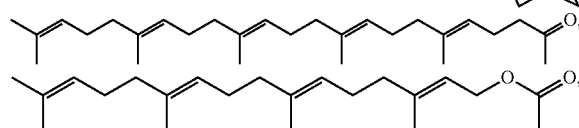
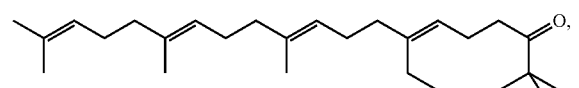
In some illustrative embodiments, CNS-G002, shows activity below the promotion threshold in the next assay downstream and showed no observable neuroprotective activity under the test conditions in the kainic acid rat model, Assay 4. In some illustrative embodiments, CNS-G001, while passing the HSP70 assay, did not exhibit sufficient activity under the test conditions to have warranted its being promoted to Assay 3, in which it showed that it had insufficient activity to have been of further interest, at 37% protection. Three compounds have been identified by the cascade of the first 3 assays that have protection >75% in the whole animal kainic acid model and will be considered for further study. Considering these compounds as representative, it is contemplated that the correlation between Assay 2 and the quantitative response of Assay 1b, is 78% and that Assay 3 is

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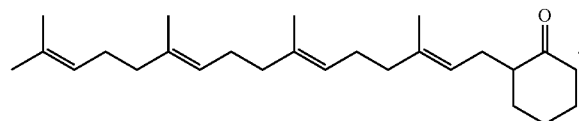
correlated 86% with Assay 2. From these results it can be reasonably concluded that the functional cascade proposed is adequate for the identification of compounds having superior performance to those that have already been identified.

The invention claimed is:

1. A method of treating amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), or ischemia, the method comprising administering to a subject in need thereof a therapeutically effective amount of a compound selected from:



and



* * * * *

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